6-19-80 **DER #7**

Fluvalinate: 13-Week Feeding Study in Rats
Zoecon Corporation. 1980. MRID No. 00077028. HED Doc. No. 001786.

82-1(a)
racemic
6-19-80

001786

Subject: 13-Week Dietary Toxicity Study in Rats. Final Report

Test Compound: ZR-3210 Technical (Racemic Mixture)
fluvalinate
(Mavrik(R)) Technical

Accession Number: 070097

Testing Facility: International Research and Development Corporation

Study Number: 322-032

Responsible Professionals:

Study Director - D. Clifford Jessup
Director of General Toxicology Division - Edwin I. Goldenthal
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Testing Period: December 6, 1979 - March 6-7, 1980

Report Submitted to Sponsor: June 19, 1980

Purity of Test Material: 93.8%

Batch or Lot Number: Analytical No. 0979-069, Run #7

Stability, Homogeneity, Concentration: The data indicated that the compound was stable and equally distributed in the feed for the respective concentrations. All concentrations were within acceptable limits.

Materials and Methods: The compound was formulated in the diet at dosage levels of 0 (corn oil alone, vehicle control), 20, 100, 250, 500, and 1500 ppm. The appropriate amount of test material was weighed and added to an equal amount of corn oil and stirred until homogeneous. This solution was mixed for 5 minutes in a Hobart blender with 500 grams of ground Certified Rodent Chow #5002 (Ralson Purina Company). The resultant premix was then added to additional ground Certified Rodent Chow #5002 and mixed in a twin shell blender for 30 minutes to yield 12 kg of prepared test diet. Starting with week 2, the intensifier bar was used for the first two minutes and last two minutes of each blending period. Fresh diets were prepared weekly, with each test diet analyzed in duplicate for the test material concentrations. Analytical values were obtained for study weeks 1-4, 8, and 13.

Additionally, the IRDC drinking water supply was analyzed. Water analysis at quarterly calender year intervals have been conducted since 1979. Samples were analyzed for heavy metals, pesticides, and coliform bacteria. The water analysis results are appended.

One hundred thirty-five male and 135 female weanling Charles River CD(R) rats were shipped from the Charles River Breeding Laboratories, Inc., Portage, Michigan and acclimated to the test facilities for 10 days prior to study initiation. Animals showing external abnormalities were culled. One hundred twenty animals of each sex were then randomly selected and assigned to one of six groups. Each group was composed of 20 animals of each sex. Weight distributions were homogeneous within groups. Group 1 received only the vehicle. Groups 2 through 6 inclusive received the dietary test compound at the respective levels of 20, 100, 250, 500, and 1500 ppm.

Rats were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity-, and light-controlled environment. All animals were uniquely identified by a metal ear tag. Ear tags were verified when rats were transferred to clean cages, as well as before and after blood and urine collection and at necropsy.

Animals were observed twice daily, 7 days a week, for signs of overt toxicity, moribundity, and mortality. Detailed observations were recorded weekly. Mortality and overt signs of toxicity were recorded on the day observed. Individual body weights were measured and recorded weekly beginning with the pre-test period. Individual food consumption was measured and recorded once during the pre-test period and weekly during the study. Mean compound consumption was calculated weekly.

A neurotoxicity screen was also conducted during the course of the study at 4, 8, and 12 weeks. A panel of 8 behavioral tests was given to 10 randomly selected rats per sex from Groups 1 (vehicle controls), 3 (100 ppm), and 6 (1500 ppm). The parameters tested included toe pinch, tail pinch, righting reflex, alley progression, locomotor activity, wire maneuver, grip strength, and Preyer's reflex.

Baseline (pre-test) clinical laboratory tests were conducted on an additional group of 10 male and 10 female rats, which were subsequently sacrificed by carbon dioxide inhalation. Ten animals/sex/group were randomly selected for hematology and biochemistry at the six-week interval. The remaining rats within each group were bled for cholinesterase analysis. Because several deaths occurred prior to 6 weeks in the 1500 ppm group, 8 males and 3 females were sampled for cholinesterase at this interval. At all other intervals 10 rats/sex/group were randomly chosen to measure each set of parameters.

Blood was obtained via puncture of the orbital sinus plexus. Urine was collected overnight from the rats while in stainless steel metabolism cages. Food and water were withheld prior to the collection of blood and urine samples. Clinical chemistry data was obtained for the following parameters: Hematology: hematocrit, hemoglobin, erythrocyte count, total leucocyte count, platelet count, reiculocyte count, mean corpuscular - volume, - hemoglobin, - hemoglobin concentration, and differential leucocyte count. Biochemistry: glucose, BUN, SGOT, SGPT, AP, albumin, total protein, calcium, cholesterol, total bilirubin, creatinine, LDH, phosphorous, sodium, potassium, chloride, globulin, cholinesterase (plasma and erythrocyte cholinesterase were measured at Weeks 6 and 13; brain cholinesterase was also measured at Week 13). Urinalysis: volume, specific gravity, color and appearance, microscopic examination of sediment, pH, protein, glucose, ketones, bilirubin, occult blood, nitrite, and urobilinogen.

An opthalmoscopic examination was also performed by a veterinary opthalmologist on all rats six days prior to the initiation of the study and 3 or 4 days prior to termination of the experiment (a description of the examination can be found within the referenced accession).

Following the terminal eye exam, 20 males and 20 females were selected for a viral screen. The selected animals included 10 per sex who showed no ocular pathology and 10 per sex showed positive ocular pathology upon examination. Priority selection was given to the control and high-dose group, where possible. Mid-dose animals were chosen when other groups could not accomodate the numbers. A serum sample was collected from each animal and heat inactivated for half an hour. The sample was then diluted 1:5 with sterile saline solution and frozen. All samples were shipped in dry ice to Microbiological Associates in Bethesda, Maryland for analysis. Viruses included in the screen were:

Pneumonic Virus of Mice
Encephalomyelitis
Toolen H-1
Mouse Adenovirus
Rat Coronavirus
Lymphocytic Choriomeningitis
Sialodacryoadenitis
Reovirus Type 3
Sendai
Mouse Hepatitis
Kilham Rat Virus

All statistical analysis compared the treatment group with the control group by sex. Body weights (Week 13), hematological, biochemical, and urinalysis parameters (Weeks 6 and 13) and absolute and relative (to both body weights and brain weight) organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-Test (for equal or unequal variances) as described by Steel and Torrie. Dunnett's multiple comparison tables were used to judge significance of differences.

All surviving rats from each group were sacrificed by carbon dioxide inhalation and necropsied at the completion of the study. This method was also used throughout the study for animals sacrificed in extremis. An examination of the external body surface, orifaces, and internal organs was conducted at necropsy. Deviations from normal were recorded. Organs and tissues were weighed fresh at necropsy and included: liver, kidneys, testes/ovaries, brain (with stem), and heart. All tissues from each rat, including all tissue(s) with gross abnormalities, were collected for fixation in buffered neutral 10% formalin. All animals sacrificed in extremis were treated similarly.

Hematoxylin and eosin stained paraffin sections of the following tissues were prepared by standard histologic technique for microscopic evaluation from all rats in the control (0 ppm) and high dose (1500 ppm) groups:

all gross lesions
adrenals (both)
trachea
eye (placed in Bouin's fixative)
esophagus
stomach
duodenum

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jejunum
ileum
cecum
pancreas
brain (3 levels - forebrain, midbrain, and hindbrain)
heart
lungs and main stem bronchi (lungs were inflated with formalin via the trachea)
pituitary
thyroid and parathyroid
thymus
lymph node (mesenteric)
sternum (bone marro
colon
liver (2 sections)
spleen
urinary bladder (inflated with formalin and left unopened for examination and fixation)
testes/ovaries
spinal cord (cervical, thoracic, and lumbar)
salivary gland (submaxillary and sublingual)
skeletal muscle (thigh)
kidneys (both)
prostate/corpus and cervix uteri
peripheral nerve (sciatic)
any other tissue(s) with lesions

In addition, sections of the heart, liver, kidneys, skeletal muscle, skin, brain, and spinal cord and sciatic nerve at 20-, 100-, 250-, and 500- ppm dosage levels were also examined microscopically.

Results: Weekly observations revealed no palpable masses for any animal during the 13-week study. The sign of excessive salivation appeared in the high-dose (1500 ppm) group during the first week of the study and disappeared by the third week of the study. Three of the five females and one of the eight males showing this sign died or were sacrificed during the first month of the study. Other signs reported to be distributed equally between control and treated groups were corneal opacities, red material around the eyes, and hair loss. Behavioral changes were not observed. Skin lesions in the form of scabs were noted as early as the first week of the study. Many of the scabs were reportedly transient. All scabs were located on the head and shoulder area. The number of animals showing scabs at week one (single or multiple) at particular dose levels is noted below:

Dosage Level (ppm)	Number of Animals Showing Scabbing	
	Males	Females
0	0	0
20	1	1
100	7	2
250	9	1
500	12	10
1500	7	7

A compound-related effect on mortality was observed in the high-dose group. Seven females and two males in the 1500 ppm group died or were sacrificed in extremis before week 4 of the study. No other deaths occurred throughout the rest of the study.

Group mean body weights recorded here as a percent change from controls are reported below:

	MALES		
	Time (Weeks)		
Dose (ppm)	2	6	13
0	-	-	-
20	-1.4	-2.5	-1.1
100	-0.4	+0.3	+2.8
250	-2.8	-5.5	-3.6
500	-7.0	-8.5	-7.9
1500	-31.5	-21.6	-18.8*
	FEMALES		
0	-	-	-
20	-2.5	-2.2	-0.8
100	+5.0	+4.0	+4.6
250	+1.9	+0.4	+2.3
500	-2.5	-4.0	-1.9
1500	-14.5	-8.1	-10.4**

*p <0.01
**p <0.05

Males showed a dose-related decrease in mean body weights at all time periods at doses of 250, 500, and 1500 ppm. However, only the high-dose group showed a statistically significant body weight decrease (-18%) at 13 weeks. Values in the high-dose group were significantly lower, although not statistically lower, than controls by 32 and 22 percent at 2 and 6 weeks respectively. Males in the 500 ppm dosage group showed a 7.0 percent relative decrease in body weight by week 2 which remained relatively constant over the remainder of the test period. Females of the 1500 ppm group showed a statistically significant decrease in mean body weight (-10.4%) when compared to controls at 13 weeks. Values were also lower by 14.5 and 8.1 percent at 2 and 6 weeks respectively but were not statistically significant. It is noted here that the dose response which was evident in males at 250, 500, and 1500 ppm was only seen in females at 1500 ppm.

Mean food with compound consumption (reported here as a percent difference from control group) and calculated mean compound consumption (mg/kg/day) for the 13-week period were as follows:

Mean Food With Compound Consumption

Dose (ppm)	Males	Females
0	-	-
20	-2.0	+3.2
100	+4.8	+5.9
250	-3.2	+1.1
500	-5.6	0.0
1500	-17.5	-13.0

Mean Compound Consumption (mg/kg/day)

Dose (ppm)	Males	Females
0	-	-
20	1.48	1.82 (+0.34)
100	7.70	8.84 (+1.14)
250	18.80	21.20 (+2.40)
500	38.00	44.30 (+6.30)
1500	116.00	124.00 (+8.00)

Males and females receiving 100 ppm of compound in the diet showed a slight increase in the values for mean food with compound consumption when compared to the control group at 13 weeks (note here the slight gains or losses in mean body weights for animals receiving 100 ppm). A dosage-related decrease of food with compound consumption was evident in males at 250, 500, and 1500 ppm, with only the decrease at 1500 ppm (-17.5%) apparently biologically significant. Values for females were comparable to controls at 250 and 500 ppm. However, the decrease at 1500 ppm (-13.0%) was considered biologically significant.

The mean food consumption, minus the compound consumption values, and reported here as the percent difference from the control group, for weeks 2, 6, and 13 were as follows:

MALES

Dose (ppm)	Time (Weeks) 2	6	13
0	-	-	-
20	-2.4	-1.8	-2.0
100	0.0	-5.2	+4.4
250	-4.1	-6.3	-4.0
500	-8.6	-7.0	-3.2
1500	-35.5	-11.8	-10.5

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	FEMALES		
0	-	-	-
20	-1.6	+1.1	+4.0
100	+3.7	+7.4	+5.8
250	+1.1	+2.6	+2.3
500	-2.7	+2.1	+8.1
1500	-19.1	-6.3	-8.1

The above values indicated that the food consumption was dramatically decreased at the 1500 ppm dose level for both males and females at week two. Males receiving 500 ppm of the test compound in the diet also showed a significant decrease in food consumption at week two. This was not, however, true for females.

It would therefore appear that the differences observed between groups receiving the test compound and those receiving vehicle alone are meaningful only in the high-dose (1500 ppm) group.

The results of clinical laboratory studies conducted on hematology, chemistry, and urinalysis were as follows:

Hematology: Males at 6 weeks:

Dose (ppm)	RBC	Hb	HCT	Reticulocytes
100	-	-	-	-
250		* d	* d	
500		* d	* d	
1500	*d	** d	** d	*1/I

* decrease; p <0.05; d
** decrease; p <0.01; d
*1/increase; p <0.05; I

A downtrend in the values for RBC, Hb, and HCT appeared to be evident beginning with 100 ppm with definitive indications at 250 ppm. An uptrend in the reticulocyte count appeared evident beginning with the 250 ppm dose level. Values for MCV, MCH, and MCHC were comparable to controls at this time period.

Males at 13 Weeks:

decreased RBC count at 1500 ppm - not statistically significant

decreased Hb at 1500 ppm - not statistically significant

decreased HCT at 1500 ppm - not statistically significant

decreased MCHC at 1500 ppm (p <0.05)

increased reticulocytes at 1500 ppm - not statistically significant.

All other values for the parameters recorded were comparable to control values at all dose levels at 6 and 13 weeks.

Hematology: Females at 6 Weeks

Dose (ppm)	RBC	Hb	HCT	Reticulocytes
20	* d	**d	* d	
100	**d	**d	**d	
250	**d	**d	**d	
500	**d	**d	**d	
1500	**d	**d	**d	**$^{1}/_{I}$

* decrease; $p < 0.05$; d
** decrease; $p < 0.01$; d
**$^{1}/_{I}$ increase; $p < 0.05$; I

A downtrend in the values for RBC, Hb and HCT was evident beginning at 20 ppm. An uptrend in the reticulocyte count appeared beginning with the 500 ppm dose level. Values for MCV, MCH, and MCHC were comparable to controls for this time period.

Females at 13 Weeks

Dose (ppm)	RBC	Hb	HCT	MCHC
500	* d	* d	* d	* d
1500	**d	**d	**d	**d

* decrease; $p < 0.05$; d
** decrease; $p < 0.01$; d

An upward trend in the reticulocyte count appeared beginning with the 500 ppm dose. However, neither dose level (500 and 1500 ppm) showed statistically significant increased values above controls.

Values for polychromia and hypochromia were comparable to control values.

The white blood cell differential counts were comparable between groups and when compared to control values.

Chemistry: Males at 6 Weeks

Dose (ppm)	20	100	250	500	1500
glucose	I*	I**	I**		
BUN					I*
SGOT					
AP					

Chemistry: Males at 6 Weeks (cont'd)

Dose (ppm)	20	100	250	500	1500
Albumin				D*	D**
SGPT					I*
Total Protein				D*	D**
Ca			D**	D*	D**
Cholesterol					
Total Biliruhin					
Creatinine				I*	I**
LDH					
P					I*
Na				I**	
K					
Cl				I**	
Globulin					D**

I = increase
D = decrease
* = $p < 0.05$
** = $p < 0.01$

Chemistry: Males at 13 Weeks

Dose (ppm)	20	100	250	500	1500
Glucose	I*	I**			
BUN					
SGOT			I**	I**	I**
AP		D*	D**	D*	
Albumin				D*	
SGPT					
Total Protein				D**	D**
Ca		I*			
Cholesterol		D*		D**	D*
Total Bilirubin			D*	D**	D**
Creatinine					
LDH			I*	I*	
P		D*			
Na		D**	D**	D**	D**
K		D**	D*	D**	D**
Cl		D**	D**	D**	D**
Globulin				D*	D**

I = increase
D = decrease
* = p <0.05
** = p <0.01

Chemistry: Females at 6 Weeks

Dose (ppm)	20	100	250	500	1500
glucose			I*		
BUN		I**		I*	I**
SGOT					D**
AP					
Albumin					D**
SGPT					
Total Protein					D**
Ca					
Cholesterol					
Total Bilirubin			D**	D*	D*
Creatinine					
LDH					D**
P					
Na					
K					
Cl					
Globulin					D**

I = increase
D = decrease
* = $p < 0.05$
** = $p < 0.01$

Chemistry: Females at 13 Weeks

Dose (ppm)	20	100	250	500	1500
glucose		I**			D*
BUN				I*	I**
SGOT		D*	I*		
AP	I**	I*	I**		
Albumin					
SGPT					
Total Protein					
Ca			D**	D*	
Cholesterol				D*	
Total Bilirubin					D**
Creatinine					
LDH		D**	I*		D*
P	D*	D**			
Na			D**	D**	D**
K			D**	D**	D**
Cl			D**	D**	D**
Globulin				I*	

I = increase
D = decrease
* = $p < 0.05$
** = $p < 0.01$

Males - 6 weeks: Statistically significant values reflecting an increased blood glucose were observed for dose levels of 20, 100, and 250 ppm. No increases were observed at 500 and 1500 ppm. Statistically significant decreases for albumin, total protein, calcium, and globulin were observed at levels of 500 and 1500 ppm. Creatinine was statistically significantly increased at 500 and 1500 ppm. Statistically significant increases were also observed at 1500 ppm for BUN, SGPT, and phosphorous. No statistically significant differences were observed for SGOT, AP, cholesterol, total bilirubin, and LDH.

Males - 13 weeks: Statistically significant values reflecting an increased blood glucose were observed for dose levels of 20 and 100 ppm. No increases were observed at higher levels. Statistically significant decreases were observed for AP, albumin, total protein, cholesterol and globulin. Decreases in some of these parameters (AP and cholesterol) began as low as 100 ppm. Statistically significant increases were observed for SGOT at 250, 500, and 1500 ppm. LDH also showed statistically significant increases at 250 and 500 ppm. Values for BUN, SGPT, Ca, creatinine, and phosphorous were generally comparable to control values. Na, K, and Cl were all statistically significantly lower for all dose levels beginning with the 100 ppm dose level.

Females - 6 weeks: Statistically significant decreases were observed for albumin, total protein, and globulin at 1500 ppm, as well as for total bilirubin at 250, 500, and 1500 ppm. BUN was increased at 100, 500, and 1500 ppm. SGOT and LDH was decreased at 1500 ppm.

Females - 13 weeks: Glucose was statistically significantly increased at 100 ppm and decreased at 1500 ppm. Cholesterol and total bilirubin were statistically decreased at 500 and 1500 ppm respectively. Alkaline phosphatase (AP) was statistically increased at 20, 100, and 250 ppm. BUN was increased at 500 and 1500 ppm. Calcium was decreased significantly at 250 and 500 ppm. Values for creatinine, total protein, SGPT, and albumin were comparable to control values. Na, K, and Cl showed statistically significant decreases beginning at 250 ppm.

Urinalysis - 13 Weeks - Males: Males showed statistically signficantly increased volume at 100, 250, and 500 ppm concurrent with a decreased specific gravity at 250 and 500 ppm. The pH was statistically significantly increased by one pH unit above controls at 250 ppm and higher (i.e. pH increased from 6 to 7).

Urinalysis - 13 Weeks - Females: Females also showed statistically significant increases of urinary volume at 100 and 250 ppm with an attendant decrease in specific gravity at 100 and 250 ppm which was significant. The increased pH of the urine (one unit pH 6 to 7) was statistically significant and 250 ppm. The pH at 100 ppm was statistically significant but it could not be determined in which direction the shift occurred (i.e., 6 vs. 6).

All other parameters for both sexes for both time periods were comparable to controls.

Cholinesterase: Plasma and brain cholinesterase activity were comparable to control values for both sexes. Males showed an increased erythrocyte cholinesterase level at 250, 500, and 1500 at 13 weeks. Readings at 6 weeks were comparable to controls. Females showed increased erythrocyte cholinesterase

levels at 250, 500, and 1500 ppm at 6 weeks and at 1500 ppm at 13 weeks.

Opthalmoscopic Examination: Corneal opacities were first observed during week 3. No animals were placed on study with observable eye abnormalities. The following table shows the number of rats with corneal opacities at the terminal eye exam.

Dose (ppm)	Males	Females
0	10/20	4/20
20	13/20	2/20
100	9/20	2/20
250	14/19	1/20
500	5/20	2/20
1500	7/18	1/13

Viral Screen: Results indicated the presence of three viruses: Sendai virus, RCV virus and SDA virus.

Neurotoxicity Screen: Six of the parameters evaluated showed similar responses for all groups tested at all (i.e., 4, 8, and 12 Week) intervals - they were, toe pinch, tail pinch, righting reflex, alley progression locomotion, and Preyer's reflex. Females showed no functional deficits with regard to the wire maneuver or grip strength. Males, however, showed a functional deficit at 1500 ppm for both the wire maneuver and grip strength. Males also showed a functional deficit at 100 ppm for the wire maneuver only.

Organ Weights: It was reported that statistically significant mean organ weight changes occurred in a few organs at various dose levels. However, in the absence of any morphologic changes (see following paragraphs) or dose response relationhip among these tissues, the biological significance of the weight changes was uncertain.

Gross pathology: No apparent treatment-related gross necropsy changes were observed. However, a slight increase in incidence of skin lesions, such as ulcer formation, was seen primarily at the 500 and 1500 ppm dose groups of both sexes. Two skin lesions were reported for each dose level of 500 and 1500 ppm for females and four skin lesions were reported for each dose level of 500 and 1500 ppm for males.

Histopathology: No treatment-related morphological changes were observed except possibly in the skin. Other lesions described were considered to be common among rats of this age and strain, and not related to compound administration. A slight increase in the incidence of ulcerative dermatitis among male and female rats at 500 and 1500 ppm was observed. This histopathology confirmed the gross findings. No nervous system pathology was found and no tumors developed in any animals.

Discussion: Body Weight: Males at 2 weeks showed parallel log-dose responses (decreases) in mean body weights and mean food consumption (i.e., mean food consumption less compound consumption) at levels of 250, 500, and 1500 ppm. It would seem reasonable to assume that for this period the decreased body weights were attributable to a decreased food consumption resulting from unpalatability of the feed mixture (note: a palatability problem is acknowledged from the results of other studies). This can be considered a normal expectation. Measurements at 6 weeks at dose levels of 250 and 500 ppm revealed log-dose response decreases in mean body weights, while food consumption remained approximately the same at these two dose levels. These decreases in the mean percent change for body weight with attendant food consumption intake results appear to indicate a threshhold level for compound-induced toxic effects rather than weight decreases attributable primarily to decreased food consumption. At 1500 ppm, a 12% decrease in food consumption resulted in a 22% decrease in body weight gain, thereby reinforcing the assumptions made for the lower dose levels. It would therefore seem reasonable to assume that a substantial portion (if not all) of the animal weight loss could be attributed to compound. The rationale applied to the 6 week interval can also be applied to the 13-week interval. However, at 13 weeks an 11% decrease in food consumption resulted in a statistically significant decrease (19%) in mean body weight percent changes at 1500 ppm.

It therefore appears reasonable to conclude that the decreased body weights for males after 2 weeks at dose levels of 250, 500, and 1500 ppm are log-dose responsive with body weight decreases not solely attributable to decreased food consumption but rather due to compound-related toxic effects.

Discussion: Body Weight: Females at 2 weeks show a generally proportional response between food intake and mean body weight changes and probably represents animal acclimation to the test diet rather than a compound-related toxic effect. At 13 weeks and 500 ppm, an 8% increase in food consumption resulted in a 2% decrease in mean percent body weight gain and at 1500 ppm, an 8% decrease in food consumption resulted in a statistically significant decrease in the mean percent body weight change. These later changes appear to suggest toxic effects of the compound rather than decreased food consumption. These same arguments appear to be applicable for the effects observed at 6 weeks at 500 and 1500 ppm.

It seems reasonable to conclude that for males a decreased food utilization appears evident beginning at 250 ppm and for females at 500 ppm. Males responded in a log-dose response manner. A compensatory response for females to the toxic effects of the compound appeared to be an increased rate of food ingestion which was apparently not exercised in males.

It is also interesting to note here that even though less females survived than males at the high-dose level, those females that did survive appeared more resistant to the effects of compound when measured by this parameter.

Hematology: Males and females both gave strong evidence for the presence of an anemia. Females showed statistically significant decreases for Hb, HCT, and RBC count at all dose levels for the 6-week reading. A downtrend was readily evident at all dose levels. An uptrend for the recorded values of reticulocytes was evident at dose levels of 500 and 1500 ppm, with the increased number of reticulocytes being indicative of a homeostatic blood compensatory mechanism secondary to the anemia. Other supportive evidence for

the presence of anemia was reflected in the decreased values for MCHC which was a consequence of the increased presence of the reticulocytes (see also paragraph on bilirubin in chemistry section). This same line of reasoning can also be applied to the results presented for males.

The above reported effects appear to be toxic manifestations of the compound and not a normal adaptive mechanism as suggested by the author of the report. It would seem that a general "normal" adaptive mechanistic response to a chemically induced non-specific stress as suggested by the author would be supported by attendant changes in white blood cell count, larger adrenal gland, possibly smaller gonads, and other changes which one might expect to observe as a result of an induced non-specific stress.

This reviewer therefore holds the position that the hematological changes observed are not the result of adaptive changes to a non-specific stress but rather a compound specific toxic effect.

The decreased values for HCT, Hb, and RBC count at all dose levels in females at 6 weeks precludes the establishment of a NOEL for this experiment.

Clinical Chemistry: Inspection of the data for males at 6 weeks indicates compound-related toxic effects on liver function (pathology shows no morphological changes) which was definitive at levels of 500 and 1500 ppm. Albumin, total protein and globulin were all decreased. These findings for adverse liver function are reinforced at 13 weeks with decreased albumin, total protein, globulin, and cholesterol with a statistically significant decreased cholesterol level noted at 100 ppm and at higher doses. Decreased values for many of these parameters were also evident for females although not to the same extent as males. The clinical chemistry values appear to correlate well with values for percent change in body weight for both sexes as well as food consumption. Males showed decreased food intake and decreased protein serum levels and results for females also correlate well. The lower bilirubin levels also appear well correlated with the anemia for both sexes and time interval. The changes recorded for calcium are not easily explained. The increased blood glucose levels may be a result of the stress of handling. This reviewer tends to agree with the author of the report that the decreases recorded for the serum electrolytes are probably due to a dilution error. Other values appear to occur at random and may or may not be related to variation in experimental technique.

Urinalysis: No easy explanation is offered for the results noted for this parameter. However, the shift in pH may be reflective of acid metabolites formed by metabolic degradation of the parent compound, OR INCREASED FAT METABOLISM SECONDARY TO DECREASED FOOD INTAKE

The results of the urinalysis tend to support the argument for an error in dilution for the serum electrolytes (i.e., Na, K, Cl). MBK

Cholinesterase: The statistically significant increases in values for RBC cholinesterase are not readily explainable, but may not be biologically meaningful. However, hemolysis during in vitro testing should not be ruled out.

Opthalmoscopic Examination: The number of animals exhibiting corneal disease was unusually high and the distribution overwhelmingly in the males. There was no obvious explanation for this distribution. In most instances it

appeared to be a chronic problem that was in a static or healing period as evidenced by the presence of most vessels and large chronic vascular beds. Also there was no active conjunctivitis observed. There was no progression of involvement in the dosage groups and the significant numbers involving the controls would suggest that this disease was not compound-related.

Neurotoxicity Screen: The high dose group males showed an apparent change in response to the wire maneuver and grip strength tests at the four-week interval as compared to the control. This response might be correlated with the toxic effects observed in the high-dose group during the initial weeks of the study. The biological significance of this finding is not known.

Viral Screen: These results indicated the presence of three viruses in the rats in this study. Sendai virus and RCV will manifest themselves as upper respiratory infections, while SDA will produce a transient conjunctivitis and corneal opacity along with a swelling of the salivary glands. While no mention was made of swollen necks in the weekly observations, it is possible that the corneal opacities seen in these rats could have been virally induced by SDA or another unknown virus.

Direct correlation between viral titer and eye pathology could not be made, possibly because the disease was not active when the blood was analyzed, but was in a static or healing period as suggested at the terminal eye exam. Since the opacities were observed equally in the control and test groups, they were not considered compound induced.

The significance of the predominantly male distribution of the corneal lesions cannot be explained.

Pathology: The general absence of morphological changes appears to reinforce the hypothesis that the test compound affects liver function without obvious structural damage.

Classification: Core - ~~Guideline~~ minimum *CB*

LEL: 20 ppm females decreased HCT, Hb, RBC count (i.e., anemia)

NOEL: Less than 20 ppm. No NOEL was established.

The following comment was made during the course of the review.

(1) Please supply references for all studies in the neurotoxic screen.